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Drab6 and the secretory pathway affect oocyte polarity in *Drosophila*

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SUMMARY

The *Drosophila* oocyte is a highly polarized cell. Secretion occurs towards restricted neighbouring cells and asymmetric transport controls the localization of several mRNAs to distinct cortical compartments. Here, we describe a role for the *Drosophila* ortholog of the Rab6 GTPase, Drab6, in establishing cell polarity during oogenesis. We found that Drab6 localizes to Golgi and Golgi-derived membranes and interacts with BicD. We also provide evidence that Drab6 and BicD function together to ensure the correct delivery of secretory pathway components, such as the TGF- α homolog Gurken, to the plasma membrane. Moreover, in the absence of Drab6, *osk* mRNA localization and the organization of microtubule plus ends at the posterior of the oocyte were both severely affected. Our results point to a possible connection between Rab protein-mediated secretion, organization of the cytoskeleton and mRNA transport.

INTRODUCTION

By regulating the transport of proteins and lipids toward the plasma membrane, the secretory pathway plays an important function in cell polarity. Rab GTPases are important regulatory factors of vesicular traffic. Members of the Rab6 family regulate protein transport between the Golgi, endoplasmic reticulum, plasma membrane and endosome (Del Nery et al., 2006; Mallard et al., 2002; Martinez et al., 1997; Martinez et al., 1994; Opdam et al., 2000). The role of Rab6 in establishing cell polarity was, however, unclear. We chose the *Drosophila* oocyte as a model to study cell polarity *in vivo*. The oocyte lies at the posterior of the egg chamber, which consists of a cluster of 16 interconnected germ cells surrounded by a monolayer of follicular epithelium. During egg chamber formation, the germ line forms a sixteen cell cluster and in which one cell is singled out to become the oocyte, while its 15 sister cells develop into nurse cells (for review, see (Huynh and St Johnston, 2004)). During early oogenesis, microtubules (MTs) are nucleated from the microtubule-organizing center at the posterior of the oocyte. Towards stage 7, an unidentified signal from the posterior follicle cells triggers the organization of perpendicular MT subsets controlling the dorsoventral axis (DV) and anteroposterior axis (AP) in the oocyte (Januschke et al., 2006; MacDougall et al., 2003). *Bicoid*, *oskar* and *gurken* mRNAs, which determine the embryonic axes, are then localized, respectively, to the anterior, posterior and antero-dorsal poles of the oocyte (Riechmann and Ephrussi, 2001).

Studies in cultured mammalian cells have revealed a molecular mechanism whereby BicD modulates MT based Golgi trafficking by recruiting cytoplasmic Dynein to transport vesicles (Hoogenraad et al., 2001; Matanis et al., 2002). The recruitment of Dynein to vesicles is mediated by the interaction of BicD with the small Golgi localized GTPase Rab6 (Matanis et al., 2002; Short et al., 2005). In *Drosophila*, and more specifically in the oocyte, the Golgi

apparatus is not organized into stacked cisternae arranged into “Golgi ribbons”. Instead, it is organized into mini stacks of tER-Golgi units evenly distributed throughout the cell (Herpers and Rabouille, 2004; Kondylis et al., 2001). Whereas three Rab6 isoforms have been characterized in mammals, (Del Nery et al., 2006; Mallard et al., 2002; Martinez et al., 1997; Martinez et al., 1994; Opdam et al., 2000), only one has been identified in *Drosophila* (Shetty et al., 1998). So far, Drab6 has been shown to be involved in Rhodopsin transport in photoreceptor cells and bristle morphogenesis (Purcell and Artavanis-Tsakonas, 1999; Shetty et al., 1998). In this study, we present the characterization of the function of Drab6 during oogenesis and propose a possible connection between Rab protein-mediated secretion, the organization of the cytoskeleton and mRNA transport.

Materials and Methods

Fly stocks

w¹¹¹⁸ used as wild-type; *rab6^{D23D}* (Purcell and Artavanis-Tsakonas, 1999) was recombined to FRT-40A (Bloomington). *rab6^{D23D}* and *khc^{7.288}* germ line and follicle cells clones were generated as described in (Januschke *et al.* 2002). *GFP-trap*, *GalT* and *PDI* (A. Debec). *Khc-LacZ* (I. Clark). *BicD-GFP* and *BicD^{mom}* (B. Suter). Over-expression of *Dynamitin* and Colchicine treatment was performed as described in (Januschke *et al.* 2002).

Transgenes, biochemistry and immunohistochemistry.

Drab6 was cloned into maternally expressed *tubGFP* (Januschke *et al.* 2002) and Polyubiquitin mRFP vectors. Details of protocols for biochemistry experiments can be obtained upon request. Yeast two-hybrid screen was carried out as described (Formstecher *et al.* 2005).

Electron microscopy and immunodetections were performed as described in (Januschke *et al.*, 2006). Antibodies: Stau (St Johnston *et al.*, 1991); Osk (Hachet and Ephrussi, 2001); β -Galactosidase (Roche); Grk (DSHB); Lav (W. Sullivan); BicD, Syntaxin-5 (DSHB); KDEL (Stressgen); Dynactin (EL. Holzbaaur); GFP (Roche). LE Lectin (Vector). WGA, Phalloidin, Lysotracker (Molecular Probe).

RESULTS AND DISCUSSION

In vertebrate cells, Rab6 is associated with the Golgi and the *trans*-Golgi network (TGN) membranes (Del Nery et al., 2006; Mallard et al., 2002; Martinez et al., 1997; Martinez et al., 1994; Opdam et al., 2000). To investigate the sub-cellular localization of Drab6 in the *Drosophila* germline, we monitored the expression pattern of transgenic lines expressing Drab6 fused to GFP (Fig. 1A) and RFP (Fig. 1B). We observed that during oogenesis, the global distribution of Drab6 evolved. Drab6 first accumulated transiently in a central position during stages 7/8, then was uniformly distributed at the beginning of stage 9 to end up juxtaposed to the entire oocyte cortex (Fig. 1A). It is noteworthy that promoters of different strengths gave similar expression patterns. In addition, the genomic null allele *rab6*^{D23D} (Purcell and Artavanis-Tsakonas, 1999) was fully rescued by the different lines expressing Drab6.

Drab6 did not co-localize extensively with ER membranes (labelled with PDI-GFP, Bobinnec et al., 2003) (Fig. 1B). Instead, it seemed to be differentially associated with two types of Golgi unit (Fig. 1C). Lava Lamp (Lav), a *cis*-Golgi marker (Papoulas et al., 2005) co-localized with Drab6, mainly at the cortex of the oocyte and in nurse cells. A GFP trap protein coding for an UDP-galactose beta-N-acetylglucosamine beta-1,3-galactosyltransferase (GalT) (Morin et al., 2001), predominantly enriched in Golgi membranes (Fig. 1D), exhibited a distribution similar to that of GFP-Drab6: it accumulated in the center of the oocyte at stage 8, where it co-localized with Drab6, and was later confined to the cortex (Fig. 1C). Importantly, the distribution of Lav and GalT was similar in both *mat α tubGFP-Drab6*, *ubiRFP-Drab6* and control oocytes (Fig. S3). Given that Lav and GalT markers are not present in the Golgi cisternae that are evenly distributed throughout the oocyte, as documented by electron microscopy (EM) analysis (Herpers and Rabouille, 2004), they may be the hallmark of distinct functional Golgi units, with Drab6 being able to interact with both of them. Unlike

Lav, whose distribution is only mildly affected (Fig. 1C), GalT and acetyl-glucosamin modified proteins (detected by the wheat germ agglutinin lectin (WGA)) expressed by Golgi structures were abnormally distributed in *Drab6* mutants (Fig. 1C). Moreover, ultrastructural analysis with EM in *Drab6* mutant oocytes revealed that the ER was abnormally swollen (Fig. 2 A' vs B'), and that the Golgi mini-stacks were markedly curved with partially inflated cisternae (Fig. 2 A" vs B").

These morphological effects led us to investigate the role of Drab6 in the secretory pathway. We monitored the polarized secretion of the TGF α -like growth factor Grk (Neuman-Silberberg and Schuepbach, 1993). Grk secretion is restricted to the antero-dorsal corner through a rapid transit from the ER towards the Golgi apparatus (Herpers and Rabouille 2004). In GFP-Drab6 rescued egg chambers, Grk and Drab6 co-localized (Fig. 2C). In *Drab6* mutant oocytes, *grk* mRNA localization is the same as in wild type (Fig. S1). The protein, however, was slightly more abundant than in controls and an important fraction extended ventrally (Fig. 2E). Polarized secretion of Grk led to the formation of two dorsal appendages on the egg shell (Fig. 2D). In the absence of Drab6, mislocalized Grk induced ventralization, (Fig. 2E, inset, 22 % absent dorsal appendages, 28 % fused, $n=199$), instead of a dorsalization (multiple dorsal appendages on the egg shell) as observed when Grk is ectopically secreted (Neuman-Silberberg and Schuepbach, 1994). Hence, this argues for a specific failure of Grk delivery to the plasma membrane. This phenotype is specific to Drab6 since it could be fully rescued by the GFP-Drab6 transgene (Fig. 2C).

Next, we analysed the intracellular localization of Grk in absence of Drab6. Grk accumulated frequently in large ring-like particles in the *Drab6* mutant but not in control oocytes (Fig. 2D, E). Those Grk “rings”, similar to those of yolk granules (Bokel et al., 2006; Queenan et al., 1999) did not contain Lav (Fig. 2G), suggesting that Grk is not blocked in the Golgi. Grk actually accumulated in *Drab6* mutants on vesicles stained by LysoTracker (Fig. 2H vs. I)

which labels either lysosomes or late endosomes containing yolk granules (Dermaut et al., 2005). Hence, two independent approaches suggest that Grk is not blocked in the Golgi but is rather mis-localized to post-Golgi compartments, probably endosomes.

Interestingly, the secretory impairment was also confirmed by *Lycopersicon Esculentum* Tomato Lectin (LE), detecting modified proteins in the Golgi. In absence of Drab6, LE revealed abnormal vesicular structures in the oocyte and nurse cells that had failed to reach the cortex (Fig. S2). EM analysis also demonstrated rupture of the plasma membrane between neighbouring nurse cells (Fig. S2). Finally, we observed that GFP-Drab6 rescued egg chambers exhibited an accumulative enrichment at the plasma membrane during oogenesis, which was particularly evident in nurse cells (Fig. S2). This is consistent with the involvement of Drab6 in secretion towards the plasmalemma.

We have established the existence of three important and novel aspects of Drab6 function during oogenesis:

- i) Consistent with its localization in vertebrate cells, Drab6 is predominantly localized to the Golgi complex in *Drosophila*, but overlaps with Golgi markers that have distinct localizations, suggesting that Drab6 may associate with distinct functional Golgi units. Drab6 might also play a role in membrane exchange between Golgi and ER and in Golgi organization according to our EM analysis, which is again consistent with known functions of mammalian Rab6 (Del Nery et al., 2006; Martinez et al., 1997; Young et al., 2005).
- ii) By controlling the migration of Golgi units towards the cell cortex, Drab6 controls the delivery of membrane to the plasmalemma as shown in *Drab6* mutants in which glycosylated proteins labelled by WGA and LE Lectins accumulate in large vesicular structures. This pattern is similar to the mis-localization profile of Grk in the absence of Drab6.
- iii) In the oocyte, Drab6 is required for the antero-dorsal secretion of Grk, which leads to the differentiation of the follicle cells required for the morphogenesis of the dorsal appendages of

the egg shell. In the absence of Drab6, we observed that Grk is mis-localized to late endosomal or lysosomal compartments, demonstrating that Drab6 also affects post-Golgi traffic. In vertebrate cells, one of the Rab6 isoforms (Rab6A') is also involved in endosome to Golgi transport (Del Nery et al., 2006; Utskarpen et al., 2006). Additionally, a role for Ypt6p (the only copy of Rab6 in the yeast *S. cerevisiae*) has also been documented for fusion of endosome-derived vesicles with the late Golgi (Siniossoglou and Pelham, 2001). It remains to be established whether Drab6 functions directly in the secretory pathway or if the effects observed in *Drab6* mutants on post-Golgi trafficking are a consequence of defects in endosome to Golgi trafficking.

In order to identify potential Drab6 binding proteins, we performed a yeast two-hybrid screen (Formstecher et al., 2005), using as bait Drab6Q71L, a GTP-ase deficient mutant. Sixty-two distinct truncated clones of BicD, lacking parts of the amino terminus, interacted with Drab6Q71L (data not shown). The intersection of all identified fragments defined a minimal interacting domain, mapping BicD amino acids 699-772 in the coiled-coil motif H4 (Fig. 3A), shown for murine BicD to interact with the mammalian Rab6 (Matanis et al., 2002). In order to validate this interaction, we performed glutathione S-transferase (GST) pull-down assays, using lysates from wild type ovaries. GST-Drab6 specifically retained BicD, since GST alone and GST-Rab1 did not bind BicD. Furthermore, preloading GST-Drab6 with the non-hydrolyzable GTP analog, GTP- γ -S yielded an improved interaction with BicD (Fig. 3B). We conclude, therefore, that *in vitro*, BicD interacts through its carboxy terminus preferentially with the active form of Drab6 (GTP-bound), like mammalian Rab6 (Matanis et al., 2002; Short et al., 2002).

Time lapse recording showed that in the oocyte and nurse cells, RFP-Drab6 and BicD-GFP (Pare and Suter, 2000) co-localize to multiple large aggregates with low dynamics (supp. MOV2 and Fig. 3B). Further GFP-Drab6 accumulation in the center depended on the

presence of BicD during stage 8 as observed in a *BicD^{mom}* background (see Swan and Suter, 1996, Fig. 3C). Interestingly, in such *BicD^{mom}* oocytes, Grk was found in ring-like structures remote from the nucleus as in Drab6 mutant oocytes (Fig. 3C).

Since BicD and Rab6 have been shown to be involved in MT-based transport, we checked whether Drab6 positive structures require MTs to move. Time lapse microscopy revealed that: big aggregates were less dynamic than the highly motile small particles. Colchicine MT depolymerization severely reduced the movement of Drab6 particles movements which formed big clusters (Fig. 4B), indicating that Drab6 is actively transported along MT. The MT motors Kinesin I and Dynein have been shown to be involved in polarizing the *Drosophila* oocyte (Brendza et al., 2000; Brendza et al., 2002; Duncan and Warrior, 2002; Januschke et al., 2002). Inactivating the Dynein complex by the over-expression of Dynamitin, (Januschke et al. 2002) prevented accumulation of Drab6 at the oocyte cortex (Fig. 4C) but did not significantly reduce Drab6 movements (Fig. 4C'). In contrast, in *khc^{7.288}* germ line clones, Drab6 did not localize in the centre of the oocyte during stage 7/8 and formed abnormal aggregates around the mispositioned nucleus. For reasons we currently do not fully understand, the speed of Drab6 particles was significantly reduced when compared to controls or over-expressing Dynamitin oocytes (Fig. 4D).

We observed that Drab6 and BicD interact in a yeast two hybrid screen and in GST pull down assays and co-localize *in vivo*. Moreover, there were indications that Drab6 requires BicD for correct sub-cellular localization, which suggests that Drab6 interacts with BicD in *Drosophila* as it does in mammals. Strikingly, we found that lack of both proteins compromises Grk secretion in a very similar way. Overexpression of Dynamitin, to impair Dynein function, induces ectopic accumulation of Grk and ventralization of the egg shell as

well (Januschke et al. 2002). Therefore, in *Drosophila*, BicD/Dynein and Drab6 are likely involved together in Grk secretion to the antero dorsal corner of the oocyte.

It is important to mention that co-localization of the two proteins was limited. Moreover lack of BicD or Drab6 yields two different phenotypes. BicD mutation affects oocyte determination and the position of the oocyte nucleus (Swan et al., 1999), but has no impact on MT organization in mid-oogenesis (Swan and Suter, 1996), which is not the case in the *Drab6* mutant (Fig. S1). A genetic interaction between BicD's co-factor Egalitarian and Kinesin I has already been demonstrated (Navarro et al., 2004), hence suggesting that Drab6 may interact with Dynein and Kinesin I via BicD.

Interestingly, we noticed that, in absence of Drab6, *oskar* mRNA was not correctly localized in the oocyte (Fig. S1) (42% dot, 31% diffuse, 10% undetectable, remainder wild-type, $n=75$). *gurken* and *bicoid* mRNAs were, however, unaffected (Fig. S1), and *osk* mRNA localization to the oocyte center is frequent when the MT network is not correctly polarized (St Johnston, 2005). In *Drab6* mutant oocytes, the defective posterior localization of the MT plus end marker Khc- β Gal (Clark et al., 1997) indicates a defect in MT organization (Fig. S1). Similar observations have been recently reported (Coutelis and Ephrussi, 2007).

Given that Drab6 is required for late Grk signaling at the anterodorsal corner of the oocyte, it might also be involved in early germ line to soma signaling mediated by Grk, which controls MT organization (Gonzalez-Reyes et al., 1995; Roth et al., 1995). Nevertheless, we think that this is unlikely. In absence of this signaling, posterior follicle cells differentiate into anterior follicle cells, and, as a consequence, the posterior structure of the eggshell, the aeropyle, is substituted with an anterior structure, the micropyle (Gonzalez-Reyes et al., 1995; Roth et al., 1995). We always observed an aeropyle at the posterior of eggs derived from *Drab6* mutant oocytes (Fig. S2). Additionally, removing Drab6 from the posterior follicle cells did not affect oocyte polarity. Drab6 is, hence, possibly involved in MT organization at the posterior pole.

Interestingly, Rab6 family interactors such as Rab6IP2/ELKS (Monier et al., 2002) are capable of interacting with CLASPs at the cortex of HeLa cells (Lansbergen et al., 2006) suggesting a link between Rab6 protein and MT organization at the cortex.

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Figure legends

Figure 1. *Drab6 shows a dynamic localization and is enriched on Golgi membranes.*

(A) Drab6 mutant oocytes rescued by GFP-Drab6 expression showed a stage-dependent distribution: Drab6 was central during stages 7 and 8 (arrow, 40%, $n=112$), uniform during stage 9 (86%, $n=81$), and always juxtaposed to the oocyte cortex from the end of stage 9 on ($n=64$). (B) RFP-Drab6 and PDI-GFP co-expressing egg chamber. (C) Co-localization of Drab6 and effects of its loss on different Golgi markers in oocytes. Lav (I) co-localized with Drab6 in a rescued egg chamber mainly at the cortex (II, arrows), but global Lav localization did not depend on Drab6 (III). (VI) GalT co-localized with RFP-Drab6 in the centre during stage 8 (V, arrow; inset stage 10 egg chamber), but did not accumulate in the centre in *rab6^{D23D}* (VI). WGA was central during stage 8 (VII, arrow), co-localized with GalT (VIII, arrow) but formed abnormal ring-like aggregates in *rab6^{D23D}* (IX, arrow in inset, inset magnified view of boxed area). (D) Immunoblots of fractions from a membrane density gradient of GalT expressing ovaries tested with markers specific to the Golgi (Dynactin), the ER (KDEL and Syntaxin 5) and the plasma membrane (Syntaxin 5). GalT was predominantly enriched in fractions containing Golgi membranes, but was additionally found in fractions reflecting the plasma membrane. Vertical bars on the left indicate the sedimentation profile: ER, endoplasmatic reticulum; PM, plasma membrane. Scale bar, 20 μ m.

Figure 2. *Grk is mis-localized to post Golgi compartments in Drab6 mutants.*

(A, B) Electron micrographs, low magnification of control and mutant egg chamber, respectively (magnified areas highlighted. *nc*, nurse cells; *oo*, oocyte; *fc*, follicle cells). ER morphology in control (arrows in A') and in *rab6^{D23D}* (arrows in B'). Normal tER-Golgi unit morphology in the control (A'') and onion-like shaped morphology in mutant (B''), cisternae

(arrowheads) were not altered in number, although severely swollen). (C) Drab6 and Grk co-localization in GFP-Drab6 rescued oocyte. (D, E) Projection of optical sections ($\sim 20\mu\text{m}$) of control and *rab6^{D23D}* stage 9 oocytes, stained for Grk and F-actin. Upper insets, confocal sections at the nucleus. Lower insets, control egg and ventralized egg in the mutant. In contrast to the control, Grk formed larger particles close to and ring-like structures remote from the nucleus (arrowhead) in the mutant. (F) Grk and Lav co-localized marginally to small particles close to the nucleus in the control and in *rab6^{D23D}* oocytes (G). Co-localization was not observed for the large Grk positive ring-like structures (arrowheads). (H, I) Control and Drab6 mutant egg chamber, respectively, labelled with Grk and Lysotracker (Lyso) to reveal endosomal compartments and lysosomes. (H', I') Magnified view of upper boxed area. (H'', I'') Magnified view of lower boxed area. Grk co-localized in a ring-like manner with Lyso positive structures close to and distal to the nucleus only in *rab6^{D23D}*. Asterisk, oocyte nucleus, scale bar, $20\mu\text{m}$ (500nm in EM micrographs).

Figure 3 *Drab6 interacts with BicD in Drosophila.*

(A) Schematic representation of BicD. Exemplary truncated BicD clones containing the C-terminal H4 coiled coil domain that interacted with Drab6 in the two hybrid screen. (B) Interaction of Drab6 and BicD. (I) Western blot. GST-Drab6 specifically retained BicD. Preloading GST-Drab6 with the non-hydrolyzable GTP analog, GTP- γ -S, yielded an improved interaction. In addition to BicD ($\sim 89\text{kDa}$), a polypeptide of lower molecular weight (probably a degradation product, $\sim 60\text{kDa}$) was specifically retained on GST-Drab6 beads and could be revealed with the 1B11 but not the 4C2 antibody (II). (III-V) Frames taken from time lapse recording on BicD-GFP (III) and RFP-Drab6 (IV) co-expressing egg chamber(supp. MOV1), which co-localized to several aggregates in nurse cells and the oocyte

(V). (C) Drab6 accumulates in the centre (arrow) in GFP-Drab6 rescued egg chambers (I). In *BicD^{mom}* egg chambers of equal age (II), GFP-Drab6 accumulation in the centre was abolished (arrow). In *BicD^{mom}* oocytes, Grk protein accumulated in ring-like structures remote from the nucleus (III, arrow in lower inset, i.e. magnification of boxed area). Asterisk, position of oocyte nucleus, scale bar 20µm,

Figure 4 *Drab6 is actively transported along microtubules.*

All images shown are frames taken from time lapse recordings on GFP-Drab6 expressing egg chambers. Untreated (A) GFP-Drab6 rescued egg chamber (supp. MOV3) and colchicine treated (B) egg chamber (supp. MOV4). (C) GFP-Drab6 expressing egg chamber over-expressing Dynamin (supp. MOV5) and (D) *khc^{7.288}* germ line clone expressing GFP-Drab6 (supp. MOV6). Below each panel parameters of vesicle movement derived from the time-lapse recordings are indicated. Particle parameters were determined using ImageJ. Particles in oocytes and nurse cells were traced in a single optical plane in three different egg chambers for each: control, *khc* clones and over-expression of Dynamin. (A, B) Colchicine treatment abolished accumulation in the center as seen in controls (arrow) and particles seemed to form bigger clusters. (C) Over expressing Dynamin reduced accumulation of Drab6 at the cortex (arrow). (F) Stage 8 *khc⁷⁸⁸* oocyte expressing GFP-Drab6. Drab6 did not accumulate in the center and formed clusters close to the oocyte nucleus (asterisks). Scale bar, 20µm.

Supplementary Material

Figure S1

(A) Fluorescent *osk* mRNA *in situ* hybridizations. (A) Wild type. (A') mutant oocyte (*rab6^{D23D}* germ line clone). *osk* mRNA mis-localized to the center and the oocyte nucleus. (B) Mosaic egg chamber stained for Osk protein (red). *Drab6* mutant follicle cell clones were labelled by the absence of GFP (green), and outlined with dotted lines. Osk protein accumulation to the posterior is not affected. (C) Projections of several optical sections of stage 10 egg chambers stained against Stau (red) and with Phalloidin (green). Stau localized to the posterior in wild type (C). In *Drab6* mutants, Stau accumulated in the center of the oocyte and close to the nucleus (C'). (D) Cuticle preparations of a wild type and *Drab6* mutant (D') lacking abdominal structures. (E) Egg chambers expressing Kin-βGal to label MT plus ends. Wild type (E) and *Drab6* mutant (E'). (F) Fluorescent *grk* mRNA *in situ* hybridisation. *grk* localized to the anterodorsal corner of wild type (F) and *Drab6* mutant oocytes (F'). (G) Fluorescent *bcd* mRNA *in situ* hybridization. *bcd* localization in a characteristic ring shape to the anterior cortex in wild type (G) and *Drab6* mutants (G'). (H) Grk signaling to the posterior follicle cells is unaffected in *Drab6* mutants as indicated by the presence of a posterior aeropyle. Anterior micropyles (arrows), posterior aeryples (arrowheads). Asterisk, oocyte nucleus, scale bar, 20μm.

Figure S2. *Drab6* affects plasma membrane integrity.

(A) Stage 9 egg chambers stained with LE. (A) Control. (A') *Drab6* mutant. Absence of *Drab6* leads to massive accumulation of LE positive patches in the nurse cells (arrow) and to LE labeling of ring-like structures in the oocyte (see inset, arrowhead). (B) EM to monitor integrity of plasma membranes between neighbouring nurse cells in a control egg chamber (B) and in a *Drab6* mutant (B'). Continuous membranes were observed in the control (arrow), but ruptures were frequently present in the mutant (arrow). (C) *Drab6* mutant egg chamber

stained to visualize F-actin and DNA. Inset, control stained to visualize F-actin. The strong phenotype affecting nurse cell membrane integrity also caused disorganization of the actin cytoskeleton. DAPI staining revealed that these cells were not degenerated. (D) Drab6 mutant oocytes, rescued by GFP-Drab6 expression. (D) During stage 7, Drab6 was scarcely detectable on membranes (arrow). (D') Recorded with the same confocal settings, during stage 10, Drab6 massively accumulated juxtaposed to nurse cell membranes (arrow). Scale bar, 20μm (500nm in EM micrographs).

Figure S3. GFP-Drab6 and RFP-Drab6 expression do not affect the localization of Golgi-associated proteins.

(A) Distribution of Golgi-associated proteins in stage 9 egg chambers. (I – III) Egg chambers stained with Lav, (IV, V) Egg chambers expressing GalT-GFP. (I) W^{1118} (wild type) egg chamber, (II) $Drab6^{D23D}/Cyo$; $ubi-RFP-Drab6 / Tm2$ egg chamber, (III) $Drab6^{D23D}/Cyo$; $mat\alpha tub-GFP-Drab6 / Tm2$ egg chamber, (IV) W^{1118} ; $GalT-GFP$ egg chamber, (V) $Drab6^{D23D}/GalT-GFP$; $ubi-RFP-Drab6 / Tm2$ egg chamber. The distribution of GFP-GalT was not analyzed in $mat\alpha tub-GFP-Drab6$ egg chambers, both Drab6 and GalT were tagged with GFP. In Drab6 overexpressing oocytes, the distribution of the Golgi-associated proteins Lav and GalT-GFP is similar to that observed in wild type oocytes. Scale bar, 20μm

(B) Level of expression of RFP-Drab6 and GFP-Drab6

Western blots of ovarian extracts from flies expressing different levels of Drab6: W^{1118} (wild type) ovaries, $Drab6^{D23D}/Drab6^{D23D}$; $ubi-RFP-Drab6 / Tm2$ ovaries, $Drab6^{D23D}/Cyo$; $ubi-RFP-Drab6 / Tm2$ ovaries, $Drab6^{D23D}/Cyo$; $mat\alpha tub-GFP-Drab6 / Tm2$ ovaries. (I) Western blot probes with anti DRab6 antibody. The bands at 22 kD correspond to endogenous Drab6 and the bands at 50 kD correspond to the different forms of GFP and RFP tagged Drab6. In absence of Drab6 ($Drab6^{D23D}/Drab6^{D23D}$; $ubi-RFP-Drab6 / Tm2$ ovaries) the band at 22 KD

is not detectable, confirming the specificity of the antibody for Drab6. (II) Western blot probes with anti α -Tubulin for loading control. In *Drab6^{D23D}/Cyo; ubi-RFP-Drab6 / Tm2* and *Drab6^{D23D}/ Cyo; mat α tub-GFP-Drab6 / Tm2* ovaries, the amount of Drab6 is approximately twice that in wild type (*W¹¹⁸*). Signal quantification was done with MultiGauge software (Fujifilm). We used α -Tubulin as a loading reference. Drab6 antibody (Eurogentec) was obtained from rabbit after immunization with two peptides (sequences can be obtained upon request); Rab6 antibody does not function in whole mount.

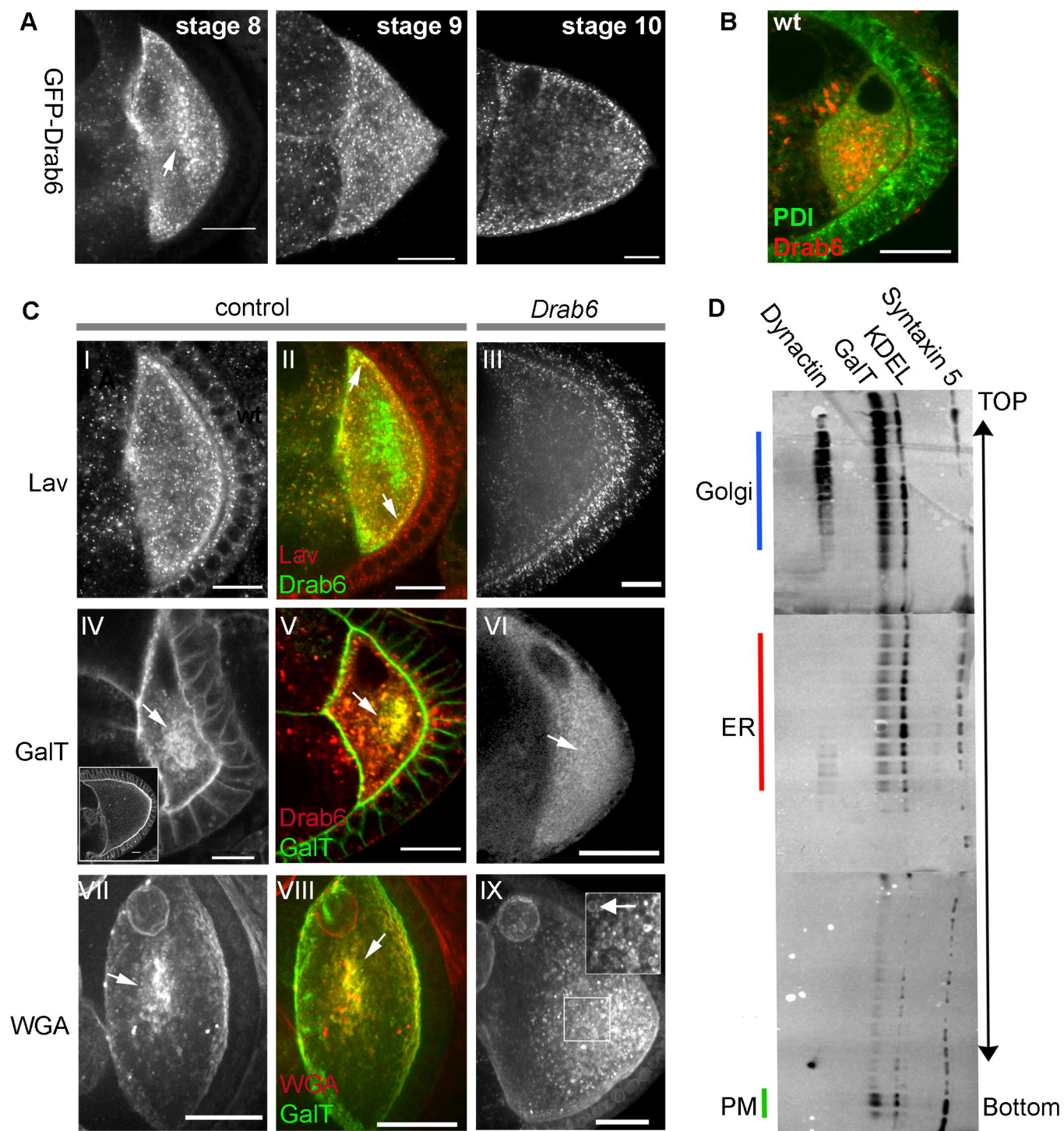


Figure 1 Januschke et al.

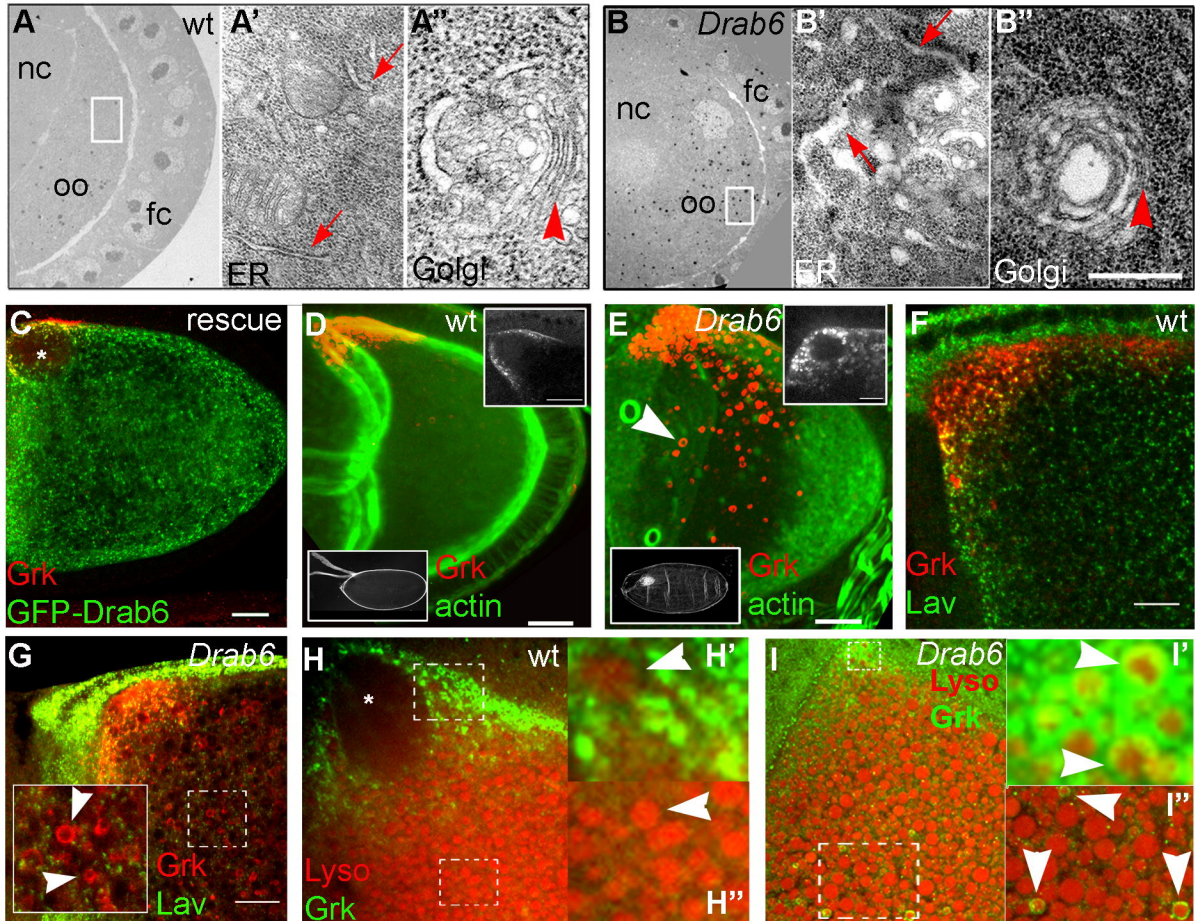


Figure 2 Januschke et al.

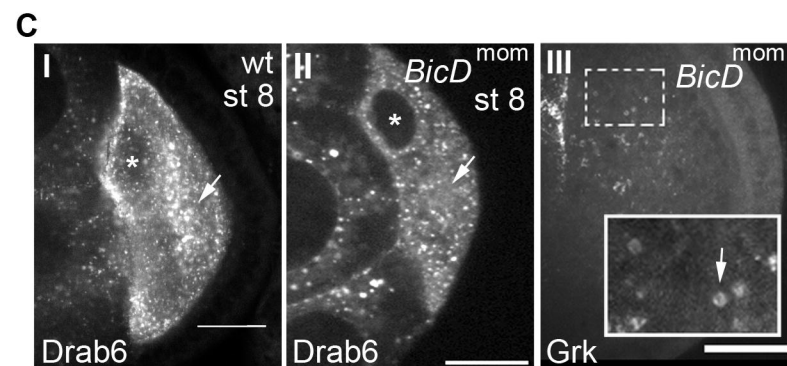
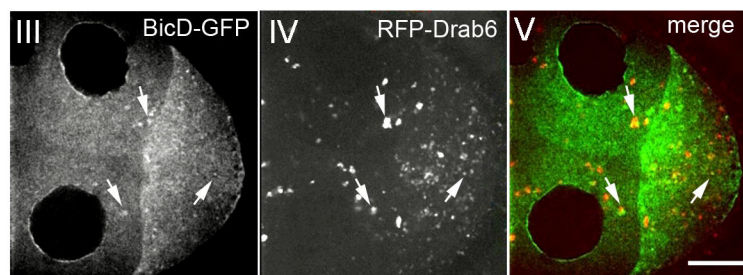
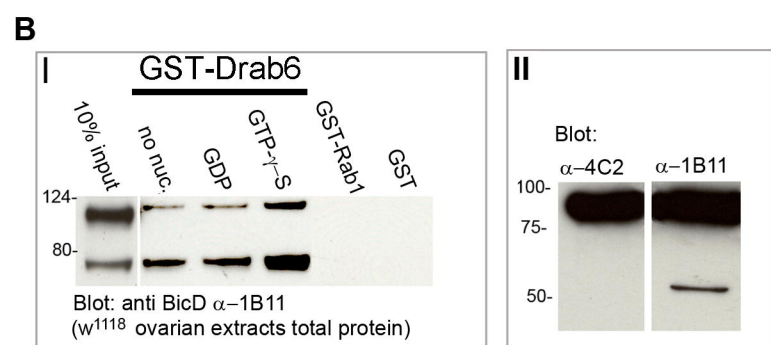
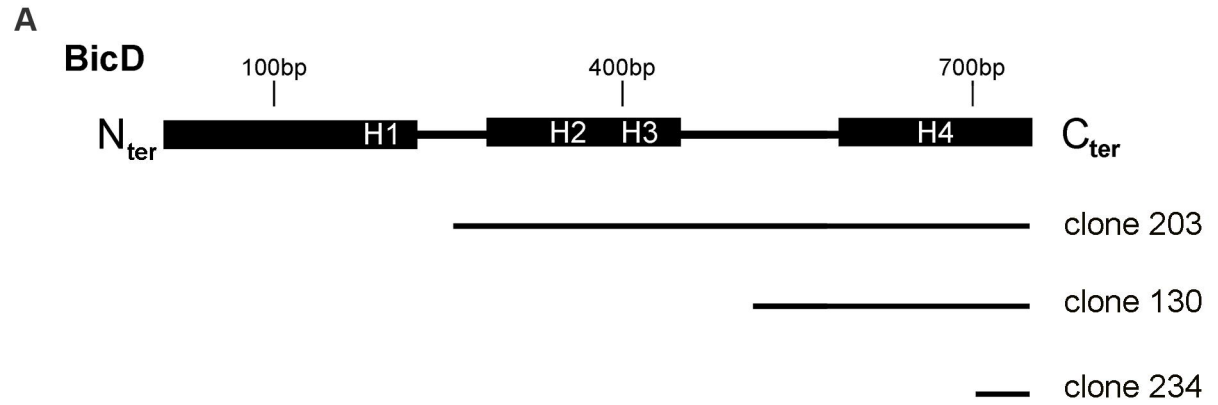
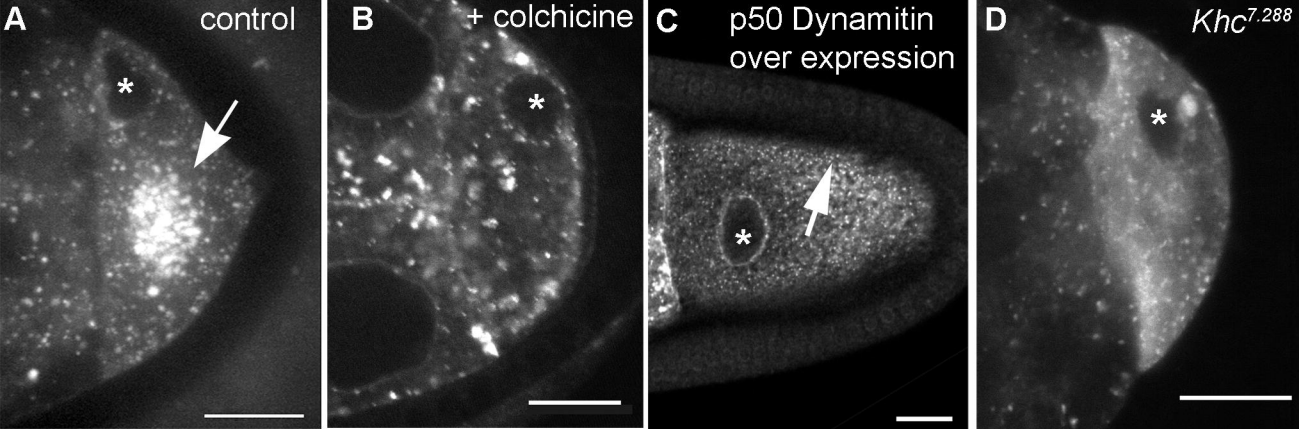


Figure 3 Januschke et al.



A' control	B' + colchicine	C' ++p50/Dynamin	D' Khc^{7.288} mutants
vesicle speed [μm/sec]±S.E.:	vesicle speed [μm/sec]±S.E.	vesicle speed [μm/sec]±S.E.	vesicle speed [μm/sec]±S.E.
0.33±0.06	0.03±0.01	0.253±0.016	0.10±0.01
(max/min):	(max/min):	(max/min):	(max/min):
0.99/0.13	0.06/0.013	0.95/0.06	0.25/0,05
n: 62	n: 51	n: 97	n: 92

Figure 4 Januschke et al.